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(54) Title: NITRIC OXIDE SYNTHASE INHIBITORS

(57) Abstract

The use of an effective amount of at least one nitric oxide synthase inhibitor in a cosmetic composition of for making a pharmaceutical composition is disclosed, said inhibitor or pharmaceutical composition being intended to reduce the skin irritant effect of topically applied cosmetic or pharmaceutical substances. A cosmetic or pharmaceutical composition containing an effective amount of at least one nitric oxide synthase inhibitor, and a cosmetic treatment method using said cosmetic composition, are also disclosed.

NO-SYNTHASE INHIBITORS

The use of an effective amount of at least one NO synthase inhibitor in a cosmetic composition of for making a pharmaceutical composition is disclosed, said inhibitor or pharmaceutical composition being intended to reduce the skin irritant effect of topically applied cosmetic or pharmaceutical substances.

A cosmetic or pharmaceutical composition containing an effective amount of at least one NO oxide synthase inhibitor, and a cosmetic treatment method using said cosmetic composition, are also disclosed.

Within the scope of this invention the skin irritant effect is a response from the skin manifesting itself in most cases by redness, pain and a prickling sensation, this response being caused by chemical products of a natural or synthetic origin applied topically to the skin. This irritation is accompanied by an alteration in the epithelial function and/or structure directly linked to the effect of the irritating product.

The disorders engendered by an irritating product are therefore followed by a fairly intense response from the skin aimed at restoring the interrupted homeostatic balance or repairing the damage caused. This response may be infraclinical, i.e. without any inflammatory reaction visible to the naked eye. However, the fairly intense reaction remains the most common tissue response to attack by an irritating product and the most annoying response for the user of said irritating product.

When the irritating product attacks the skin it may react with certain substances that already exist in the cells and tissues and/or may release intracellular substances. These released substances may in turn become active on other targets in the epithelium or derma. This therefore triggers the cascade of reactions which give rise to the irritating process characterised mainly by an irritation of the skin, this being due to the involvement of blood cells and the substances they release. This process manifests itself in particular, to varying degrees depending largely on the quality and/or quantity of the product applied and/or on the user of said product, in dysaesthetic sensations (hot flushes, sensation of burning, itching or pruritus, prickling sensations, cramp-like pains, etc.) and in redness and/or in an oedema.

These irritating products may of course be used for other effects in cosmetic or pharmaceutical compositions, and more particularly dermatological compositions. They are therefore generally used as active agents, surfactants, preservatives, perfumes, solvents or propellants with said compositions.

However, because of their irritating nature, these products are generally used in very low doses. The use of these products in a small quantity may then be considered less advantageous than the use of other less active, but less irritating or showing no irritation at all, and therefore used in a larger quantity.

Consequently there is a need in the cosmetic and pharmaceutical fields to find an method of using these products without causing irritation to the user.

Now the Applicant has discovered that NO synthase inhibitors enable the irritating nature of these products to be limited, even suppressed.

The object of this invention is therefore the use of an effective amount of at least one NO-synthase inhibitor in a cosmetic composition or for the manufacture of a pharmaceutical composition, this inhibitor or pharmaceutical composition being intended to reduce the skin irritant effect of cosmetic or pharmaceutical substances applied topically.

The cosmetic or pharmaceutical composition comprising the NO-synthase inhibitor may or may not include the product likely to cause a skin irritation.

Where these compounds are contained in the same composition, a composition for topical, cosmetic or pharmaceutical application is also disclosed, characterised in that it contains, in a cosmetically or pharmaceutically acceptable environment, an effective amount of at least one NO-synthase inhibitor and at least one product likely to cause a skin irritation.

The pharmaceutical composition is preferably a dermatological composition.

A method of cosmetic treatment, characterised in that it makes use of the cosmetic composition according to the invention, is also disclosed.

The effective quantity of at least one NO-synthase inhibitor according to the invention is a quantity of at least one NO-synthase inhibitor that is sufficient for the skin irritant effect to be reduced, even disappear. This quantity therefore varies according to the quantity and nature of the irritating product applied. By way of illustration, however, a composition according to the invention may contain at least one NO-synthase inhibitor with a concentration by weight of between 10^{-6} % and 10% of the total weight of the composition and preferably between 10^{-4} % and 1% of the total weight of the composition.

In the composition according to the invention the quantity of the product likely to cause skin irritation may therefore correspond to a quantity sufficient to cause a skin irritation if it were to be used in isolation (without the NO-synthase inhibitor).

Numerous topically applied substances have an irritating effect, particularly in people (users) with skin that is easily irritated (prone to irritation).

Therefore even products which are considered inert in a cosmetic or pharmaceutical composition, more particularly a dermatological composition, may exhibit an irritating character when they are applied to the skin, the scalp, nails or mucous membranes, these products including, in particular, preservatives, surfactants, perfumes, solvents or propellants.

Products considered to be active agents in cosmetic or pharmaceutical compositions may therefore exhibit an irritating character when applied to the skin, the scalp, nails or mucous membranes, and we may refer to this as a secondary irritant effect. These products include, in particular, certain sun blocks, the α-hydroxy acids (glycolic, lactic, malic, citric, tartaric, mandelic), the ß-hydroxy acids (salicylic acid and its derivatives), the α-keto acids, the retinoids (retinol and its esters, retinal, retinoic acid and its derivatives, retinoids, particularly those described in the documents FR-A-2 570 377, EP-A-199 36, EP-A-325 540, EP-A-402 072), the anthalines (dioxyanthranol), the anthranoids (for example, those described in the document EP-A- 319028), the peroxides (particularly benzoyl peroxide), minoxidil and its derivatives, the lithium salts, the antiproliferatives, such as 5-fluuorouracyl or methothrexate, certain vitamins such as vitamin D and its derivatives, vitamin B9 and its derivatives, capillary paints or dyes (paraphenylene

diamine and its derivatives, the aminophenols), fragrant alcoholic solutions (perfumes, eaux de toilette, after-shave, deodorants), the antiperspirant agents (certain aluminium salts), hair-removing or perming agents (thiols), depigmenting agents (hydroquinone), capsaicine, active insecticides (pyrethrin), ionic and non-ionic detergents and pro-pigmenters (dihydroxyacetone, the proralenes and the methylangecilines).

Among these products with a secondary irritant effect, the invention applies more particularly to the retinoids.

Among the retinoids particular mention may be made of all-trans retinoic acid, retinoic acid 13-b, carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophene, 6-[3-(1-adamanthyl)-4-methoxyphenyl]-e-naphthanoic acid sold under the name of Adapalene ™ by the company Galderma and Tazarotene ™ sold by the company Allergan.

Among vitamin D and its derivatives particular mention may be made of vitamin D3, vitamin D2, 1, 25-diOH vitamin d3 (calcitriol), calcipotriol, 1, 24-diOH vitamin D3 (such as tacalcitol), 24, 25-diOH vitamin D3, 1-OH vitamin D2, 1, 24-diOH vitamin D2.

Among the derivatives of salicylic acid particular mention may be made of n-octanoyl-5-salicylic acid and n-dodecanoyl-5-salicylic acid or their esters.

Nitrogen monoxide (NO) is generated enzymatically by L-arginine, the enzyme being called NO-synthase.

According to the invention the NO-synthase inhibitors are products which enable the synthesis of nitrogen monoxide (NO) to be inhibited partially, even totally, in situ in man.

This enzyme exists in two forms, the constitutive form and the inducible form (Medicine/Sciences, 1992, 8, pp. 843-845). Among the inhibitors there is a preference for using the constitutive NO-synthase inhibitors, i.e. constitutive NO-synthase inhibits as much as or more than inducible NO-synthase. The tests for identifying the constitute or inducible NO-synthase inhibitors are described in detail in the patent US 5132453.

Among these constitute NO-synthase inhibitors, preference is given to the endothelial NO-synthase.

In fact, without wanting to make a link to any theory of the invention, it appears that the reduction in the irritation observed in this invention is due mainly to the inhibition of the constitutive NO synthase, and more particularly to the inhibition of NO-synthase in the endothelial cells.

Thus among these inhibitors of constitutive NO-synthase particular mention may be made of N^G-monomethyl-L-arginine (NMMA), methylated ester of N^G-nitro-L-arginine (NAME), N^G-nitro-L-arginine (NNA), N^G-amino-L-arginine (NAA\0, N^g.N^G-dimethyl-arginine (asymmetrical dimethyl arginine, called ADMA).

Preference is given to NMMA, NAME, NNA and ADMA.

The inhibitors of NO-synthase may be used in isolation or in a mixture.

The inhibitors of NO-synthase may be used both preventively and curatively.

The particular advantage of this invention is that it is able to increase the quantity of active agents with an irritant character in cosmetic or pharmaceutical compositions

compared to the quantity normally used, in order to improve the efficiency of the latter. It is therefore possible to use hydroxy acids up to 50% of the weight of the composition, or retinoids up to 5%, without any inconvenience to the user.

The NO-synthase inhibitor or inhibitors may be applied enterally, parenterally or topically.

When applied topically, direct application to the skin, scalp, nails or mucous membranes is preferred.

The compositions according to the invention may be presented in all galenic forms, and these compositions are prepared by the usual methods.

A cosmetically or dermatologically acceptable environment is generally an environment that is compatible with the skin, the scalp, the nails or the mucous membranes. The composition containing the NO-synthase inhibitor may therefore be applied to the face, the neck, the hair and nails, or to any other cutaneous area of the body (axillary, sub-mammary regions, creases in the elbow, etc.).

When applied topically, the compositions according to the invention are presented mainly in the form of aqueous, hydroalcoholic or oily solutions, dispersions of the lotion or serum type, anhydrous or lipophilic gels, emulsions of a liquid or semi-liquid consistency of the milk type obtained by dispersion from a fatty to an aqueous phase (H/E), or vice versa (E/H), or of suspensions or emulsions of a soft, semi-solid or solid consistency of the cream or gel type, or even of microemulsions, microcapsules, microparticles or vesicular dispersions of the ionic and/or non-ionic type. These compositions are prepared by the usual methods.

When applied enterally, the compositions according to the invention may be presented in the form of tablets, capsules, coated tablets, syrups, suspensions, solutions, powders, granules, emulsions, microspheres or nanospheres, or lipidic or polymeric vesicles allowing controlled release.

When applied parenterally the compositions may be presented in the for of solutions or suspensions for perfusion or injection.

They may also be used for the scalp in the form of aqueous, alcoholic or hydroalcoholic solutions, or in the form of creams, gels, emulsions, mousses or even in the form of compositions for aerosols that also contain a propellant under pressure.

The quantities of the different constituents of the compositions according to the invention are those used as standard in the fields considered.

These compositions include, in particular, shaving mousses, cleaning, protection, treatment and care creams for the face, hands, feet, the major anatomical folds in the skin and for the body (e.g. day creams, night creams, make-up removing (cleansing) creams, fluid make-ups, sun creams) fluid foundations, cleansing milks, protective or care body lotions, sun lotions, artificial tanning creams, compositions for the bath, deodorant compositions containing a bactericidal agent, after-shave gels or lotions, hair-removing creams, compositions against insect bites, analgesic compositions or compositions for treating certain skin diseases such as those mentioned above.

The compositions according to the invention may also consist of solid preparations containing soaps or cleansing bars.

The compositions may also be conditioned in the form of a composition for aerosols also containing a propellant under pressure.

The NO-synthase inhibitors may also be incorporated in different compositions for hair care or treatment, particularly shampoos, possibly anti-parasitic, setting lotions, treating lotions, hair creams or gels, dye compositions (particularly oxidation dyes), possibly in the form of colouring shampoos, restructuring hair lotions, perming compositions (particularly compositions for the first time the hair is permed), lotions or gels preventing hair-loss, etc.

The compositions of the invention may also be used for oral/dental applications, for example a toothpaste or a mouthwash. In this case the compositions may contain normal adjuvants and additives for the compositions for oral application, particularly surfactants, thickening agents, moisturising agents, polishing agents such as silicon, various active ingredients such as fluorides, particularly sodium fluoride, and possibly sweetening agents such as sodium saccharinate.

When the composition of the invention is a solution, the proportion of the fatty phase may range from 5% to 80% by weight, and preferably 5% to 50% by weight in relation to the total weight of the composition. The oils, emulsifiers and co-emulsifiers used in the composition in the form of emulsion are selected from those used as standard in the cosmetic and pharmaceutical fields The emulsifier and co-emulsifier are present in the composition in a proportion ranging from 0.3% to 30% by weight, and preferably from 0.5 to 30%, or better 0.5 to 20% by weight in relation to the total weight of the composition. Moreover, the emulsion may contain lipidic vesicles.

When the composition according to the invention is an oily solution or gel, the fatty phase may represent over 90% of the total weight of the composition.

According to prior art the composition of the invention may also contain the adjuvants normally used in the cosmetic or pharmaceutical fields, such as hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active agents, preservatives, antioxidants, solvents, perfumes, charges, filters, bactericides, odour absorbents and colouring materials. The quantities of these different adjuvants are those used as standard in the cosmetic or pharmaceutical fields, for example from 0.01% to 10% of the total weight of the composition. These adjuvants may, according to their nature, be introduced into the fatty phase, the aqueous phase and/or into the lipidic vesicles.

Among oils that can be used in the invention mention may be made of the mineral oils (Vaseline oil), vegetable oils (liquid fraction of shea butter, sunflower oil), animal oils (perhydrosqualene), synthetic oils (Purcellin oil), siliconised oils (cyclomethicone) and fluoridated oils (perfluoropolyethers). Fatty alcohols, fatty acids (stearic acid),and waxes (paraffin, carnaub, beeswax) may also be used as fatty materials.

Among the emulsifiers that may be used in the invention, mention may be made, for example, of glycerine stearate, polysorbate 60 and the mixture of PEG-6/PEG-32/Glycol Stearate sold under the name of Tefose^{R} 63 by the company Gattefosse.

Among solvents that may be used in the invention mention may be made of the lower alcohols, particularly ethanol and isopropanol, and propylene glycol.

Among the hydrophilic agents mention may be made of the carboxyvinyl polymers (carbomer), the acrylic polymers, such as acrylate/alkylacrylate copolymers, the polyacryl amides, the polysaccharides such as hydroxypropyl cellulose, natural gums and clays, and among the lipophilic gelling agents mention may be made of the modified clays such

as bentones, metal salts of fatty acids such as the aluminium stearates and hydrophobic silicon, or even ethyl cellulose and polyethylene.

Among the hydrophilic active agents use may be made of proteins or protein hydrolysates, amino acids, polyols, urea, allantoin, sugars and sugar derivatives, hydrosoluble vitamins, starch and vegetable extracts, particularly Aloe Vera extracts.

Among lipophilic active agents use may be made of retinol (vitamin A) and its derivatives, tocopherol (vitamin E) and its derivatives, essential fatty acids, essential fatty acids, ceramides and essential oils.

Among other things it is possible to combine the NO-synthase inhibitors with active agents intended primarily for the prevention and/or treatment of skin diseases, and among these active agents mention may be made, by way of example, of

- agents modulating the differentiation and/or proliferation and/or skin pigmentation, such as the retinoids, vitamin D and its derivatives, the oestrogens, such as oestradiol, kojic acid or hydroquinone;
- bactericidal agents such as clindamycin phosphate, erythromycin or antibiotics in the tetracycline class;
- anti—parasitic agents, particularly metronidazole, crotamitone or the pyrethrinoids;
- the fungicides, particularly the compounds belonging to the imidazole class, such as econoazole, ketoconazole or miconazole, or their salts, the polyenic compounds such as amphotericine B, the compounds of the allyl amine family, such as terbinafine, or even octopirox;
- the steroidal anti-inflammatory agents such as hydrocortisone, betamethasone valerate or clobetasol propionate, or the non-steroidal anti-inflammatory agents such as ibuprofen and its salts, diclofenac and its salts, acetyl salicylic acid, acetaminophene or glycyrrhetinic acid;
- anaesthetic agents such as lidocaine chlorohydrate and its derivatives;
- antipruriginous agents such as thenaldine, trimeprazine or cyproheptadine;
- antiviral agents such as acyclovir;
- keratolytic agents such as alpha- and beta-hydroxycarboxylic or beta-ketocarboxylic acids, their salts, amides or esters, and more particularly the alpha-hydroxy acids such as glycolic acid, lactic acid, tartaric acid, citric acid, and generally the acids of fruits and the beta-hydroxy acids such as salicylic acid and its derivatives, particularly alkoylated derivatives, such as n-octanoyl-5-salicylic acid;
- the free anti-radical agents such as alpha-tocopherol or its esters, the superoxide dismutases, certain chelating agents of metals or ascorbic acid and its esters;
- antiseborrheics, such as progesterone;
- antipellicular agents such as octopirox or zinc pyrithione;
- anti-acne agents such as retinoic acid or benzoyl peroxide.

Of course, the expert ensures that he selects any compounds present in the composition according to the invention so that the properties intrinsically associated with this invention are not altered or are not substantially altered.

The pharmaceutical compositions according to the invention are ideal for use in the following areas of treatment, these treatments being particularly well adapted when these compositions contain retinoids:

1) for treating dermatological complaints associated with a keratinisation disorder relating to differentiation and proliferation, particularly for treating common, blackheads, polymorphous and rosaceous acne, nodulocystic acnes, conglobata, senile acnes, secondary acnes such as solar acne, medicinal or professional acne;

2) for treating other types of keratinisation disorders, particularly ichtyoses, ichtyosiform conditions, Darrier's disease, palmoplantar kerotodermias, leucoplasias and leucoplasiform conditions, cutaneous lichen of the (oral) mucous membrane;

3) for treating other dermatological complaints associated with a keratinisation disorder with an inflammatory and/or immuno-allergic component, and in particular all forms of psoriasis, whether it be cutaneous, mucous or ungual, and even psoriatic rheumatism, or even cutaneous atopia, such as eczema or respiratory atopia, or even gingival hypertrophy; the compounds may also be used in certain inflammatory complaints not presenting a keratinisation disorder;

4) for treating all dermal or epidermal proliferations, whether benign or malignant, whether or not of viral origin, such as common verrucas, plain verrucas or verruciform epidermodysplasia, oral or florid papillomatoses, and the proliferations that may be induced by ultraviolet radiation, particularly in the case of baso- and spinocellular epitheliomas;

5) for treating other dermatological disorders such as bullate dermatoses and collagenic diseases;

6) for treating certain ophthalmological disorders, particularly corneopathies;

7) for repairing or controlling ageing of the skin, whether photo-induced or chronological, or for reducing pigmentations and actinic keratoses, or any pathologies associated with chronological or actinic ageing;

8) for preventing or curing stigmata of epidermal and/or dermal atrophy induced by local or systemic corticosteroids, or any other form of cutaneous atrophy;

9) for preventing or treating scarring disorders of for preventing or repairing stretch marks;

10) for controlling disorders of the sebaceous function, such as acne hyperseborrhoea or simple acne or seborrhoea;

11) in the treatment of or prevention of cancerous or pre-cancerous conditions;

12) in the treatment of inflammatory complaints such as arthritis;

13) in the treatment of any complaint of viral origin affecting the skin or generally;

14) in the prevention or treatment of alopecia;

15) in the treatment of dermatological or general disorders with an immunological component;

16) in the treatment of disorders of the cardiovascular system, such as arteriosclerosis.

A further object of this invention is a method for cosmetic treatment, characterised in that it makes use of the cosmetic composition according to the invention.

The cosmetic treatment method should preferably consist in applying to the skin, scalp and/or the mucous membranes a composition such as that described above.

The cosmetic treatment method according to the invention may be used in particular by applying the hygienic or cosmetic compositions such as those defined above, according to the normal method of using these compositions. For example: application of creams, gels, sera, lotions, cleaning milks or after-sun compositions to the skin or to dry hair, application of a hair lotion to wet hair shampoos or even the application of toothpaste on the gums.

In the cosmetic field, the compositions according to the invention are ideally suited, because of the active agents contained in this composition, for use in body and hair hygiene, and particularly for the treatment of skins showing a tendency to acne, for hair regrowth, for hair-loss prevention, for controlling the greasy appearance of skin or hair, for counteracting the harmful effects of the sun and/or for controlling photo-induced or chronological ageing.

Several examples will now be given, by way of illustration and without any limitation, for obtaining active compounds with formula (I) according to the invention, as well as various specific formulations based on these compounds.

EXAMPLE 1

The purpose of this example is to demonstrate the oral *in vivo* anti-irritant activity of the methylated ester in N^G-nitro-L-arginine used as a curative agent.

The test used to evaluate this activity is the mouse ear oedema test (Balb/C strain) induced by the topical application of carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophen, at 0.01% by weight. According to this model, the response to a topical application of carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophen to the ear manifests itself in an increase in the thickness of the ear, which reaches its maximum value 5 days after application. This increase in the thickness of the mouse ear appears to be due to an increase in the thickness of the epidermis and to the occurrence of a dermal oedema. This response may therefore be easily measured using apparatus such as the oditest.

The exact operating protocol is as follows: 10 mice are first of all treated with the active product having an irritant effect, then 20 µl of an acetone solution containing 0.01% by weight of carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophen applied to one of their ears at a time t=0. The methylated ester of N^G-nitro-L-arginine is absorbed in drinking water from time t=0, with oral application to 5 (= group 2) of the 10 mice thus treated, once a day for 11 days (concentration of methylated ester of N^g-nitro-L-arginine of 1 mg/ml, i.e. 170 ± 40 mg/kg per day). The 5 mice that have not absorbed the methylated ester of N^G-nitro-L-arginine constitute group 1. The oedematous response is quantified by measuring the thickness of the ear. The results are then expressed as a percentage increase in the thickness of the mouse ear relative to the increase in the thickness observed on the other ear which had only been treated (under the same conditions as above) with an acetone solution without active agent (control or reference ear).

The results obtained are as follows:
After 5 days of treatment, the increase in the thickness of the mouse ear is at its maximum (100%) for group 1 and 70% for group 2.

The above results clearly demonstrate an inhibition of 30% of the oedema in the ear for the mice treated with this NO-synthase inhibitor.

Moreover, no sign of toxicity was observed and the weight development was not altered in the mice treated with this inhibitor.

EXAMPLE 2

The purpose of this example is to demonstrate the topical *in vivo* anti-irritant activity of N^GN^G-dimethyl aginine administered as a preventative treatment.

The test used to evaluate this activity is the same as that used in example 1.

The exact operating protocol is as follows: 5 mice are first of all treated with a gel containing as the sole active agent N^GN^G-dimethyl arginine at 1% by weight, and then proceeding with one topical application per day to one of their ears for 4 days. No increase in the thickness of the ears of the mice thus treated is observed. 20 µl of an acetone solution containing carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophen, at 0.01% by weight, is then applied topically to the ears of these 5 mice previously treated with N^GN^G-dimethyl arginine (group A) and to the ears of the untreated mice (group B), at a time t=0. The oedematous response is quantified by measuring the thickness of the ear. The results are then expressed as a percentage increase in the thickness of the mouse ear in relation to the increase in thickness observed on the other ear which had only been treated (under the same conditions as above) with an acetone solution without an active agent (control or reference ear or oedema).

By comparing groups A and B, the results obtained are as follows:
The N_GN^G-dimehyl aginine topically applied once a day for 4 days, before application of the product having an irritant effect (carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophen), reduces the amplitude by 24% and the area below the curve for the response induced by the product with the irritant effect by 50% (the curve corresponding to the thickness of the ear plotted against the days of reading).

EXAMPLE 3

The purpose of this example is to demonstrate the topical *in vivo* anti-irritant activity of N^Gmonomethyl-L-arginine (L-NMMA) used as a curative agent.

The test used to evaluate this activity is the same as that used in example 1.

The exact operating protocol is as follows: 10 mice are first of all treated with the active product having an irritant effect by proceeding with topical application to one of their ears, at a time t=0, of 20 µl of an acetone solution containing 0.01% by weight of carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophen. A gel, containing L-NMMA at 1% by weight is then applied topically to 5 (= group 2) of the 10 mice thus treated 6 hours after application of the irritant product, once a day for 5 days. The 5 mice that had not been treated with L-NMMA constitute group 1. The oedematous response is quantified by measuring the thickness of the ear. The results are then expressed as a percentage increase in the thickness of the mouse ear in relation to the increase in thickness observed on the other ear which had only been treated (under the same conditions as above) with an acetone solution without an active agent (control or reference ear or oedema).

The results obtained are as follows:
After 5 days of treatment the increase in thickness of the mouse ear is at its maximum (100%) for group 1 and 72% for group 2.

The results ther fore demonstrate an inhibition of 28% of the oedema of the ear for the mice treated with this NO-synthase inhibitor.

L-NMMA reduces the area below the curve for the response induced by the irritant product by 51% (the curve corresponding to the thickness of the ear plotted against the days of reading).

If the same treatment is given by applying betaine at 1% or 5%, or N^GN^G-dimethyl-L-arginine at 1% (symmetrical dimethyl-L-arginine, known as SDMA), instead of L-NMMA, an inhibition of 9, 16 and 7% of the ear oedema respectively is observed for the mice treated with these products, which are not NO-synthase inhibitors (see in particular for SDMA: The Lancet, Vol. 339: 572-575). A reduction by 24, 13 and 27% respectively of the area underneath the curve for the response inducted by the irritant product is also observed (the curve corresponding to the thickness of the ear plotted against the days of reading).

EXAMPLE 4

Compositions according to the invention, presented in the form of a lotion, a gel or cream for topical application, are illustrated here.

LOTION

	% by weight
Disodium EDTA	0.1
Poloxamer 182	0.2
Water	q.s.p. 100
Ethoxy diglycol	5
N^GN^G dimethyl arginine	1

GEL

	% by weight
Disodium EDTA	0.1
Poloxamer 182	0.2
Water	q.s.p. 100
Sepigel 305 sold by Seppic	3
Ethoxy diglycol	5
N^GN^G dimethyl arginine	1

CREAM

	% by weight
Disodium EDTA	0.1
Poloxamer 182	0.2
Water	q.s.p. 100
Preservatives	0.3
Sepigel 305 sold by Seppic	3
Apricot stone oil	10
Cyclomethicone	5
Ethoxy diglycol	5

Methylated ster of N^G-nitro-L-arginine		1

CREAM
oily mulsion in water

		% by weight
N^G-monomethyl-L-arginine (NMMA)		10^{-2}
Glycerine stearate		2.00
Polysorbate 60 (Tween 60 sold by ICI)		1.00
Stearic acid		1.40
Triethanolamine		0.70
Carbomer		0.40
Liquid fraction of shea butter		12.00
Perhydrosqualene		12.00
Antioxidant		0.05
Perfume		0.50
Preservative		0.30
Water	qsp	100

LOTION

Adapalene ™	0.010 G
N^9-monomethyl-L-arginine (NMMA)	0.100g
Polyethylene glycol (PEG 400)	69.890 g
95% ethanol	30.000 g

CLAIMS

1. Use of an effective quantity of at least one NO-synthase inhibitor in a cosmetic composition or for the manufacture of a pharmaceutical composition, this inhibitor or pharmaceutical composition being intended to reduce the skin irritant effect of products applied topically in the cosmetic or pharmaceutical field.

2. Use according to the preceding claim, characterised in that at least one NO-synthase inhibitor is used in a concentration by weight of between 10^{-6} % and 10% of the total eight of the composition, and preferably between 10^{-4} % and 1% of the total weight of the composition.

3. Use according to one of the preceding claims, characterised in that the irritant product applied topically to the skin, the scalp, nails or mucous membranes, is a compound selected from the preservatives of surfactants, perfumes, solvents or propellants.

4. Use according to one of claims 1 to 2, characterised in that the irritant product applied topically to the skin, the scalp, nails or mucous membranes, is a compound selected from among certain sun blocks, the α-hydroxy acids, ß-hydroxy acids, such as salicylic acid and its derivatives, the α-keto acids, ß-keto acids, the retinoids, anthralines, anthranoids, peroxides, minoxidil and its derivatives, lithium salts, the antiproliferatives, vitamin D and its derivatives, vitamin B9 and its derivatives, hair dyes or colouring agents, capsaicine, fragrant alcoholic solutions, antiperspirant agents, hair-removing or perming agents, depigmenting agents, active insecticides, detergents and pro-pigmenting agents.

5. Use according to the preceding claim, characterised in that the irritant product is selected from among the retinoids.

6. Use according to the preceding claim, characterised in that the retinoids are selected from among all-trans retinoic acid, retinoic acid 13b, carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophene, 6-[3-(1-adamanthyl)-4-methoxyphenyl]-2-naphthanoic acid, Tazarotene ™.

7. Use according to claim 4, characterised in that vitamin D and its derivatives are selected from among vitamin D3, vitamin D2, 1, 25-diOH vitamin D3 (calcitriol), calcipotriol, 1, 24-diOH vitamin D3 (such as tacalcitol), 24, 25-diOH vitamin D3, 1-OH vitamin D2, 1, 24-diOH vitamin D2.

8. Use according to claim 4, characterised in that the derivatives of salicylic acid are selected from among n-octanoyl-5-salicylic acid and n-dodecanoyl-5-salicylic acid or their esters.

9. Use according to any of the preceding claims, characterised in that the NO-synthase inhibitors are the inhibitors of constitutive NO-synthase inhibitors.

10. Use according to the preceding claim, characterised in that the constitutive NO-synthase inhibitors are the endothelial NO-synthase inhibitors.

11. Use according to one of claims 9 or 10, characterised in that the NO-synthase inhibitors are selected from among N^G-monomethyl-L-arginine (NMMA), the methylated ester of N_G-nitro-L-arginine (NAME), N^G-nitro-L-arginine (NNA), $N^G.N^G$-amino-L-arginine ()NAA), $N^G.N^G$-dimethyl arginine (asymmetrical dimethyl arginine, called ADMA).

12. Use according to the preceding claim, characterised in that the NO-synthase inhibitors are selected from among the methyl ester of N^G-nitro-L-arginine, N^G.N^G-dimethyl-arginine, N^G-nitro-L-arginine and N^G-monomethyl-L-arginine (NMMA).

13. Use according to any of the preceding claims, characterised in that the NO-synthase inhibitors are used in isolation or in a mixture.

14. Use according to any of the preceding claims, characterised in that the NO-synthase inhibitor is applied topically.

15. Composition for topical, cosmetic or pharmaceutical application, characterised in that it contains, in a cosmetically or pharmaceutically acceptable environment, of an effective quantity of at least one NO-synthase inhibitor and at least one product likely to cause skin irritation.

16. Composition according to the preceding claim, characterised in that the pharmaceutical composition is a dermatological composition.

17. Composition according to one of claims 15 or 16, characterised in that it contains at least one NO-synthase inhibitor at a concentration by weight of between 10^{-6} % and 10 % of the total weight of the composition, and preferably between 10^{-4} % and 1% of the total weight of the composition.

18. Composition according to one of claims 15 to 17, characterised in that the product likely to cause skin irritation is selected from among preservatives, surfactants, perfumes, solvents or propellants.

19. Composition according to one of claims 15 to 18, characterised in that the product likely to cause a skin irritation is selected from among sun blocks, the α-hydroxy acids, ß-hydroxy acids, such as salicylic acid and its derivatives, the α-keto acids, ß-keto acids, the retinoids, anthralines, anthranoids, peroxides, minoxidil and its derivatives, lithium salts, the antiproliferatives, vitamin D and its derivatives, vitamin B9 and its derivatives, hair dyes or colouring agents, capsaicine, fragrant alcoholic solutions, antiperspirant agents, hair-removing or perming agents, depigmenting agents, active insecticides, detergents and pro-pigmenting agents.

20. Composition according to the preceding claim, characterised in that the product likely to cause a skin irritation is selected from among the retinoids.

21. Composition according to the preceding claim, characterised in that the retinoids are selected from among al-trans retinoic acid, retinoic, retinoic acid 13b, carboxylic acid 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2naphthyl)-6-benzo[b]thiophene, 6-[3-(1-adamanthyl)-4-methoxyphenyl]-2-naphthanoic acid, Tazarotene ™.

22. Composition according to claim 19, characterised in that vitamin D and its derivatives are selected from among vitamin D3, vitamin D2, 1, 25-diOH vitamin D3 (calcitriol), calcipotriol, 1, 24-diOH vitamin D3, as tacalcitol, 24, 25-diOH vitamin D3, 1-OH vitamin D2 and 1, 24-diOH vitamin D2.

22.

23. Composition according to claim 19, characterised in that the derivatives of salicylic acid are selected from among n-octanoyl-5-salicylic acid and n-dodecanoyl-5-salicylic acid and their derivatives.

24. Composition according to one of claims 15 to 23, characterised in that the NO-synthase inhibitors are the constitutive NO-synthase inhibitors.

25. Composition according to the preceding claim, characterised in that the constitutive NO-synthase inhibitors are the endothelial NO-synthase inhibitors.

26. Composition according to one of claims 24 or 25, characterised in that the NO-synthase inhibitors are selected from among N^G-monomethyl-L-arginine (NMMA), the methylated ester of N^G-nitro-L-arginine (NAME), N^g-nitro-L-arginine (NNA), N^G-amino-L-arginine (NAA), $N^G.N^G$-dimethyl-aginine (asymmetrical dimethyl arginine, called ADMA).

27. Composition according to the preceding claim, characterised in that the NO-synthase inhibitors are selected from the methyl ester of N^G-nitro-L-arginine, $N^G.N^G$-dimethyl-arginine, N^G-nitro-L-arginine and N^G-monomethyl-L-arginine (NMMA).

28. Composition according to any of claims 15 to 17, characterised in that the NO-synthase inhibitors are used in isolation or in a mixture.

29. Composition according to any of claims 15 to 28, characterised in that it is formulated so that it can be applied to the skin, the scalp and/or the mucous membranes.

30. Method of cosmetic treatment, characterised in that it makes use of the cosmetic composition according to one of claims 15 to 29.

INTERNATIONAL SEARCH REPORT

[In English, with French translation]